EXPERIMENTAL STUDY OF TRYPANOSOMIASIS IN PALESTINE

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I. EXPERIMENTAL TRANSMISSION, SYMPTOMS AND PATHOLOGY

There is little information extant regarding the prevalence of trypanosomiasis in Palestine. There are no records of its existence prior to the war, but during the war trypanosomes were reported to have been found in army camels. Goldberg (1917) reports a severe epidemic in 1915, among the camels of the Turkish Army, during which 462 out of 956 camels died. The author classes the trypanosomes as *T. evansi*. Stuart (1923) reports that he found trypanosomes in a number of sick camels belonging to the army of occupation, which he also identified as *T. evansi*. There is, however, no record of this disease in native horses and mules.

Recently Dr. Deuel, a local veterinary surgeon discovered an outbreak of trypanosomes in mules on a farm in Northern Palestine. The nature of the disease was, apparently, not clear, since the veterinarians differed with regard to its character ; some claimed it was Nagana, while others classed it as Dourine ; Surra was, apparently, not suspected. In order to clear up this matter we undertook a study of the disease.

History of the outbreak. In September, 1922, an English mare and a native mule became ill with what appeared to be haemoglobinuria, but laboratory examinations established the presence of trypanosomes in the blood. In January, 1923, three more mules became ill and shortly after another one, making a total of 6 out of 15 animals. The symptoms were : emaciation, swelling of the breasts, intermittent fever, and occasionally haemoglobinuria. The diagnosis of trypanosomiasis was made and confirmed by blood films examined in the Hadassa Laboratory at Saffed, and by Dr. Stuart in the Central Government Laboratory. Three of the mules were native animals and two were imported from Syria ; the mare was bought in Saffed, but its past history was not known. All the animals, but the mare, had been on the farm two or three years prior to the outbreak of the illness ; the mare was the only animal acquired about four months before the discovery of the illness.

Transmission of the Infection. Thanks to the assistance of the Chief Veterinarian of the Department of Agriculture, we obtained two of the sick mules, one male and one female, for our studies.

The animals were brought to the laboratory yard and kept there for observation.

At the time of their arrival neither of the animals had trypanosomes in the blood. Nevertheless, blood was inoculated into two dogs and successful transmission obtained after an incubation period of seven days.

Subsequent to the first transmission experiment and prior to the appearance of parasites in the blood of the infected animals, we were advised by a local veterinarian (Dr. Freund) to try the effect of arecolin on the mobilization of trypanosomes in the peripheral circulation. The results were quite interesting. A dose of 0·05 gm. injected subcutaneously caused, almost immediately, marked salivation, defecation and urination. Forty-five minutes after the injection the blood was still negative, but after one hour trypanosomes were present in the peripheral blood. The drug had the identical effect in both mules.

After the appearance of trypanosomes in the blood, a second series of transmission experiments was made from both mules into dogs, rabbits and guinea-pigs. The blood was taken with a syringe from the jugular vein, a portion of it was mixed with 1·5 per cent. citrate solution, and varying amounts of the whole and citrated blood were inoculated intraperitoneally into animals as indicated in the table below. All of the animals showed trypanosomes in the blood after an incubation of six to eight days, with the exception of one guinea-pig in which the incubation period was eleven days.

Table I

Transmission of Trypanosomes from Infected Mules to Lower Animals

	Mule 1 (black, male)		Incubation	Mule 2 (white, female)		Incubation
	No.	Dose		No.	Dose	
Dogs	1	5 c.c. whole blood	6 days	1	5 c.c. citrated blood	7 days
				1	10 c.c. „ „	6 „
Rabbits ...	1	3 c.c. „ „	7 „	1	3 c.c. „ „	7 „
Guinea-pigs ...	2	2 c.c. „ „	7 & 8 „	2	2 c.c. „ „	8 & 11 „

Subsequent transmissions were carried on from guinea-pigs and rabbits. As a rule the virus was passed on in the same animal species: but occasionally transfers were made from rabbits to guinea-pigs and *vice-versa*. Inoculations were always made intraperitoneally with either whole or citrated blood. The strain has now been carried over a year through many generations and the disease has been observed in two mules, three dogs, and a large number of rabbits and guinea-pigs. It is, therefore, possible to give a composite picture of the experimental disease as it manifests itself in these animal species.

Incubation. In dogs the incubation period varied between five and seven days, average six days; in rabbits between six and eight days, average seven days; in guinea-pigs between four and eleven days, average seven days. These incubation periods correspond in general with those obtained by other observers with strains of *T. evansi* (Brown and Pearce, 1918).

The presence of parasites in the peripheral circulation was very irregular. In all the animals there were periodic remissions of greater or lesser duration, but without any regularity. Parasites were always more abundant in guinea-pigs and dogs than in rabbits. In the former they became particularly abundant a short time before death, while in rabbits the reverse was usually the case. Parasites were never abundant in the blood of the rabbit, and they were practically always absent before death.

Duration. The infection was invariably fatal for all animals studied, but the duration of the disease varied with different species. The duration in the mules is not known, but it was longer than six months. One dog succumbed after an illness of one-and-a-half months; the other two were treated and cured. Rabbits died usually within five to eight weeks after the appearance of parasites in the blood; young rabbits, however, weighing 700 to 800 grams, succumbed in three to four weeks. In guinea-pigs the results were anomalous—at the beginning they died after four to six weeks, but in the later passages the virulence seemed to have diminished and the animals survived twelve weeks or longer. There was apparently a decrease in virulence due to animal passage.

Clinical Symptoms. The two spontaneously infected mules which were sent to us were anaemic and emaciated. They were

weak, listless and unable to work. An intermittent oedema of the breasts and legs, were observed, but no abnormalities in the genitalia. There was intermittent fever and occasionally a mild haemoglobinuria.

In the experimental animals the clinical picture varied with the animal used. Dogs showed the effect of the infection after two weeks. They became listless, lost their appetite and had an appearance of being very ill. Guinea-pigs, on the other hand, showed hardly any signs of illness, and even when there were large numbers of trypanosomes in the blood the animals showed no loss of appetite. Only in rare cases of long standing infection a few skin lesions were noticed. In rabbits the symptoms were more striking, but the signs of the illness appeared more slowly than in dogs. The common features of the infection in all the animals were : an intermittent temperature, with appearance of parasites in the circulation during the febrile period ; a lymphocytosis, particularly shortly before and during the onset of fever ; abnormal changes in the red cells. In the rabbit and dog conjunctivitis and keratitis were constant signs, while in the guinea-pig they were rarely observed.

Blood Picture. The blood of infected animals showed typical changes. During the incubation period the blood picture and blood count remained normal, with the exception of a slight leucocytosis which occurred on the first day after the inoculation. With the appearance of signs of the infection, sometimes even one or two days before parasites were found in the circulation, there developed a leucopaenia associated with a definite lymphocytosis. At the same time there were changes in the red blood cells characteristic of progressive anaemia : polychromatosis, anysocytosis, large 'crescents' and Cabot's rings, which persisted, with some variations, throughout the course of the infection. The accompanying table (p. 442) is typical of the blood changes observed.

Pathology. The macroscopic and microscopic pictures of the disease varied with the animals used. In general, however, the infection in the dog and older rabbits resembled more closely that of the spontaneously infected mules than did the disease in guinea-pigs. In the rabbit and dog, as in the mule, the course of the disease was more chronic and there was extensive subcutaneous oedema and perivascular round cell infiltration in the various organs. In

the guinea-pig, the course of the disease was more acute and toxic in character ; often the guinea-pig died from the infection, without showing any external signs whatever, other than progressive loss of weight.

NATURE OF THE DISEASE. The evidence presented by our observations on the naturally and experimentally infected animals leads us to believe that we are dealing with Surra, and that the trypanosomes responsible for the condition belong to the *T. evansi* group. In morphology the organisms belong to the monomorphic group. They are slender, actively motile trypanosomes, 18 to 22μ long and 1·5 to 2·0μ wide. The posterior extremity is slightly pointed ; the undulating membrane is well-defined ; the flagellum

TABLE II

Blood Picture in Experimental Trypanosomiasis in Guinea-Pigs
(Inoculated October 22, 1923)

	23	24	25	26	27	28	29	30
W.B.C.	9,600	10,000	8,400	6,000	5,200	4,800	5,600	5,600
Polynuclears (%) ...	69	67	50	42	37	32	37	40
Lymphocytes (%) ...	28	30	46	55	59	63	59	55
Large mononuclears and transitionals (%) ...	3	3	4	3	4	5	4	5
Blood picture	normal	normal	anaemic 'crescents'	polychromatosis, 'crescents,' normoblasts.				
Trypanosomes	−	−	−	−	+	+	+	+

is free and fairly long—about one-third or less the length of the parasite. The nucleus is large, oval and central ; the blepharoblast is round and situated about one-quarter to one-third of the distance between the posterior end and the nucleus. The protoplasm is uniform or slightly granular.

The incubation period in experimental animals of five to seven days, the pathogenicity for the dog, rabbit and guinea-pig, the inability to transmit the infection by coitus and the picture of the spontaneous and experimental disease correspond fairly closely with that observed by other investigators in Surra.

CULTIVATION OF THE TRYPANOSOMES. Cultivation experiments

thus far have failed completely. A large variety of media have been tried, including the classic N.N.N. medium, the medium used by one of us (Kligler, 1924) in the cultivation of Leishmania, Noguchi's media and various modifications ; but the results have thus far been uniformly negative. The only result obtained was survival of trypanosomes for three days in normal rabbit serum incubated at 22° to 26° C.

II. EFFECT OF 'BAYER 205' IN EXPERIMENTAL TRYPANOSOMIASIS

Curative Power of the Drug. In our experiments we used dogs, rabbits, and guinea-pigs. Although mice are often preferred in chemo-therapeutic experiments on account of the ease with which parasites are found in the blood, the disease in the rabbit and the guinea-pig resembles more closely that in the mule than does the infection in mice ; the former are, therefore, more satisfactory for therapeutic tests. We also found that, using the thick drop method, it was not at all difficult to find the trypanosomes in the blood of rabbits and guinea pigs even if present only in small numbers. The blood picture in these animals is another valuable aid in following the course of the infection ; in the guinea-pig, at least, we have found it almost as diagnostic as the presence of parasites. Leucopaenia and anaemic elements (Crescents, Cabot's rings, etc.) are constant throughout infection, but disappear when the animal is cured. It is possible, therefore, by the combined method of thick drop examination and a study of cellular elements of the blood to follow without difficulty the course of the infection and the effect of the drug.

In testing the therapeutic value of the drug, we tried to determine the minimum single dose which will uniformly sterilise infected animals. The doses varied from 0·2 gm. to 0·04 gm. per kilo of body weight. The injections were made intraperitoneally from 5 per cent. or 10 per cent. solutions of the drug.

In the course of these experiments we treated a large series of guinea-pigs and rabbits in various stages of the illness, with varying amounts of the drug. Doses of 0·1 gm. per kilo or over sterilized all rabbits and guinea-pigs without exception. A dose of 0·05 gm. per kilo of body weight did not yield 100 per cent. of cures. Of fifteen

guinea-pigs treated with this dose two relapsed, one three days and the other fourteen days after treatment. Similarly one of five rabbits treated with this dose of the drug relapsed after eleven days. In all cases of relapses the condition of the animal rather than the duration of the illness seemed to be the important element. The two guinea-pigs had received the infection twenty-four days and the rabbit twenty days before treatment ; other animals treated at the same stage of the infection recovered completely.

It is evident from these experiments that a single dose of ' Bayer 205 ' is capable of sterilizing the blood of dogs, rabbits and guinea-pigs infected with a virulent strain of trypanosomes (*T. evansi*) with the apparent production of a permanent cure. The drug was administered at various stages of the infection, always with the same results.

Animals in the later stages of the infection, showing definite physical signs of the disease at the time of treatment, recovered completely and these signs cleared up within a few days after the drug was administered. Two of the guinea-pigs had advanced skin lesions at the time of treatment and within a few days the ulceration ceased and the wounds healed progressively. The smaller doses of the drug (0·05 gm. per kilo) were more satisfactory, despite the fact that a few of the animals relapsed, because of the practical absence of any noticeable toxic effect. The large doses gave more uniform results, but, as will be noted below, they were not far removed from the toxic dose of the drug, and often they actually produced toxic symptoms.

Toxic Effect of 'Bayer 205.'—In the course of our experiments, we have had occasion to note that the drug is highly toxic for rabbits and guinea-pigs. Several of our animals which were treated with the larger doses (0·1 to 0·2 gm. per kilo) died within a short period after the administration of the drug without any apparent clinical cause. Two rabbits died within six and twelve days and three guinea-pigs within two, six and seven days, respectively, after the injection of the drug. We, therefore, injected various doses of the drug into a series of animals in order to determine the lethal dose of the drug and the pathological changes produced in the body. A dose of 0·4 gm. per kilo of body weight injected intraperitoneally killed a medium size guinea-pig (460 gms.) in four days. Smaller

doses are not uniformly lethal ; a guinea-pig which received 0·25 gm. of the drug per kilo died seventeen days after the injection with typical pathological changes, while at the same time another guinea-pig receiving 0·3 gm. per kilo remained alive and well. These tests show that, for the guinea-pig at least, the therapeutic dose is approximately one-fifth to one-eighth of the lethal dose. But even smaller doses (0·1 to 0·2 gm. per kilo), which may have little effect on normal guinea-pigs or rabbits, may be decidedly toxic when injected into animals infected with trypanosomes.

Pathological Changes produced by the Drug.—In the infected animals it was difficult to differentiate the changes due to the infection from those caused by the drug. The infected animals which died after treatment showed a greater or lesser degree of degeneration of the kidney tubules, but this type of lesion was also characteristic of animals which died from a trypanosome infection. The changes found in the control guinea-pigs may be accepted as characteristic of the lesions produced by the drug. The principal lesion was extensive degeneration of the cells of the tubules, chiefly in the cortical region. The blood vessels were also congested. Another striking feature was a deposition of a brown pigment in the spleen. The liver was congested and there were, here and there, small areas of necrosis, but the changes were not as striking as those in the kidneys. The lesions found in the kidney taken with the reported presence of albuminuria in patients treated with ' Bayer 205 ' suggest a selected toxic affinity of the drug for the kidney tubules. Recently Duncan and Manson-Bahr (1924) reported almost identical changes in mice injected with lethal doses of the drug.

Prophylactic Effect of ' Bayer 205.'—Mayer and Zeiss (1921) emphasize the fact that ' Bayer 205 ' remains a long time in the animal body and consequently serves as a prophylactic against infection. Brumpt (1924) has made similar observations. These authors worked with mice. Kleine (1924) working with larger animals, failed to get protection against infection. We have tested the prophylactic effect of various doses of the drug in rabbits and guinea-pigs. The following are typical protocols :—

Rabbit Y. Weight 1390 gms. received on 1.7.23, intraperitoneally 0·10 gm. ' Bayer 205 ' per kilo of body weight. On 1.8.24, one month later, the rabbit received an infective dose of trypanosome blood. The blood of the rabbit remained negative. One month later this rabbit received another dose of infected blood,

and after an incubation period of 10 days, trypanosomes appeared in the circulation.

Rabbit 17. Weight 1680 gms., 0·05 gm. per kilo 'Bayer 205' given intraperitoneally, 16.12.23. This was followed after 25 days by an injection of trypanosomes. The result was negative. Sixty-three days later another infective dose given; after 15 and 18 days trypanosomes were found in the blood; since then the blood remained negative, but the animal developed all the clinical signs of chronic trypanosomiasis.

Rabbit 15. In this case the infective dose was given three days before the injection of 0·05 gm. 'Bayer 205' per kilo. Twelve days after the infection trypanosomes appeared in the circulation for one day. After that the blood remained negative, although clinical symptoms developed. Five weeks after the first appearance of trypanosomes the parasites were again found in the blood, and they persisted intermittently until the death of the rabbit.

Guinea-pig X. Weight 440 gms., received 0·2 gm. 'Bayer 205' per kilo, intraperitoneally and one month later an infective dose of trypanosomes. After a somewhat prolonged incubation period of 15 days, trypanosomes appeared in the circulation.

Guinea-pig 34. Weight 440 gms., 0·3 gm. 'Bayer 205' per kilo injected intraperitoneally on 16.11.23. After 25 days an infective dose of trypanosomes inoculated intraperitoneally. Trypanosomes found once after 36 days and thereafter the animal was normal. After 88 days the animal was reinfected a second time, but no signs of infection developed during the month following the infection. The animal died from a bile injection.

These observations indicate that the drug affords a considerable protection, and that the degree and duration of the protection varies with the amount of drug administered and the individual animal. It is noteworthy that the drug given shortly after the infection, that is, during the incubation period, did not prevent the development of the disease.

Trypanocidal Action of 'Bayer 205' in vitro.—Tests made *in vitro* to determine the trypanocidal action of this drug gave totally different results from those obtained *in vivo*. In the test tube a concentration of 1 : 100 of the drug failed to immobilize this strain of trypanosome in five to eight hours; while in the animal body 0·05 gm. per kilo (a dilution of 1 : 2000 or less) sterilized the blood of infected animals in fifteen hours.

Several modified procedures were employed in these experiments. In one method blood was drawn from an infected animal into a test tube containing a few glass beads. The blood was defibrinated and divided into small Wasserman tubes : 0·9 c.c. into the first tube and 0·5 c.c. into four or five others. 0·1 c.c. of a 10 per cent. solution of 'Bayer 205' was added to the first tube and further dilutions made by adding 0·5 c.c. from tube 1 to tube 2, etc. This gave a series

of dilution of 100, 200, 400, etc. The tubes were kept at 25° C. and drops examined under the dark field microscope at various intervals. This was the method of choice, because the trypanosomes remained in a favourable medium and the conditions approximated those in the animal body.

Another method consisted in the use of infected citrated plasma from which the red cells had been removed by centrifugalization for five to six minutes at 500 revolutions per second. By slow speed centrifugation the red cells are removed, while a sufficiently large number of actively motile trypanosomes remain in the supernatent plasma for the purpose of the experiments. The rest of the procedure was the same as with the defibrinated blood.

A third method consisted in the use of normal rabbit or guinea-pig serum, containing various dilutions of the drug to which small amounts of infected blood or serum were added.

Whichever method was employed the results were the same. During the first four to eight hours no effect was noted on the motility of the trypanosomes in dilutions of 1 : 100 or more. After twenty-four hours, active trypanosomes were still encountered in the 1 : 100 dilution, but the number was considerably less than in the control tubes. After forty-eight hours no motile trypanosomes were found in the dilution of 100 and 200, but the higher dilutions still contained actively motile trypanosomes.

Similar observations were made with bacteria. Contaminating air bacteria as well as *B. coli* multiplied actively in tubes containing 1 : 100 dilution of the drug.

It is apparent from these results that under the conditions of the experiments the drug is only slightly trypanocidal. This fact is of no particular importance in so far as the therapeutic value of the drug is concerned. It is of interest, however, in relation to the mechanism of the action of the drug. It is clear that either ' Bayer 205 ' undergoes some change in the body which renders it an effective trypanocide, or that it acts indirectly on the trypanosomes by inducing certain changes in the resistance of the host. In this connection it is noteworthy that the injection of ' Bayer 205 ' is followed by a marked increase in the total leucocyte count as well as in the large mononuclears ; in some animals this increase is very large and persistent.

III. MECHANISM OF RESISTANCE TO TRYPANOSOME INFECTION

Resistance of Cured Animals to Reinfection.—It is well-known that rats which recover spontaneously from an infection with *T. lewisi* are immune to a reinfection with that organism. It remained to determine whether recovery from an infection with a pathogenic trypanosome will also confer immunity on these animals. We realised that the analogy was not absolute, since our animals did not overcome the infection naturally as in the case of the rats. Nevertheless, we investigated this point by attempting to reinfect guinea-pigs and rabbits cured with 'Bayer 205.'

At various intervals after treatment with 'Bayer 205' the cured animals were injected with infective blood. These reinfections were repeated at various intervals under different conditions. All of the animals, without exception, showed a marked degree of resistance to reinfection, although the duration of this resistance varied. The resistance of guinea-pigs was apparently more persistent than that of rabbits. Several of the animals died after the second or third attempt at reinfection, but none of them showed any clinical signs of infection at the time. Reinfections were obtained in three rabbits out of the entire series and in these cases the resistance was apparently broken down artificially.

Table III (p. 449) gives a summary of the reinfection experiments, showing the duration and degree of the resistance in the different animals.

It is apparent from these results that infected guinea-pigs and rabbits cured with 'Bayer 205' develop a resistance to reinfection of long duration. In rabbits this resistance lasts three to four months, while in guinea-pigs it persists for longer periods.

Nature of Resistance.—The experiments recorded in Section 2 indicate that 'Bayer 205' injected into normal animals protects guinea-pigs and rabbits for a greater or lesser period, depending on the amount of the drug injected. This protection was not, however, as lasting as that observed in infected animals cured with 'Bayer 205.' In cured animals long-standing resistance develops even when treated with so small a dose as 0·05 gm. per kilo of body weight. Kleine and Fischer (1922), working with monkeys, also found that

cured animals developed a more persistent resistance to reinfection than those given a prophylactic dose of the drug.

In view of the bearing that this phenomenon may have on the general problem of immunity or resistance to protozoan infections, we attempted to examine more closely the mechanism of this resistance to reinfection in our cured animals.

Table III

Animals	Date of infection	Date of treatment	Dose per kilo	Reinfections: Days after treatment				Remarks
				1	2	3	4	
Rabbit 7	10.6.23	2.7.23	0.1	29	65	82	132*	20 days after last reinfection parasites appeared in the blood.
„ 8	25.6.23	2.7.23	0.1	29	65	—	—	22.10; 110 days after treatment animal died, when blood was drawn from heart. Infection negative.
„ 14	26.11.23	16.12.23	0.05	35	99	160*	—	After third reinfection, infection developed and followed a normal course.
Guinea-pig 1	8.4.23	27.4.23	0.2	44	76	95	131, 178	10.11; 197 days after treatment animal well.
„ 8	21.6.23	2.7.23	0.2	29	65	—	—	18.9; 78 days after treatment animal died of pneumonia.
„ 9	25.6.23	15.7.23	0.15	16	52	—	—	5.10; 82 days after treatment animal died while being bled.
„ 35	10.12.23	27.1.24	0.05	56	116	—	—	Died from extraneous cause 146 days after treatment; no infection.
„ 38	10.12.23	27.1.24	0.05	56	116	—	—	Observed 170 days, still alive and well.
„ 104	17.1.24	17.2.24	0.10	36	120	—	—	No infection, died after observation 147 days, from sunstroke.
„ 106	28.1.24	18.4.24	0.06	39	—	—	—	Alive and well, no signs of infection.

* Parasites appeared only after an injection of oil.

Humoral antibodies.—It is well known that the serum of rats which have recovered spontaneously from the infection with *T. lewisi* has the power to agglomerate trypanosomes. A similar condition has not been observed in animals infected with pathogenic trypanosomes. Levaditi and McIntosh (1910) have reported the

presence of a trypanocidal antibody in trypanosome infected animals following a pyrexial period. It appeared, therefore, of interest first of all to note whether there were any of the humoral antibodies in our cured and resistant animals. For this purpose a series of experiments were performed of which the following are examples. Each experiment was repeated at least two or three times, but only type experiments are given below :—

Experiment 1. Sera were collected from treated animals at intervals of 3 to 8 weeks after recovery. These sera were diluted with saline in various proportions up to 1 : 10 and suspensions of live trypanosomes added. The suspensions of trypanosomes were prepared as follows :—5 c.c. of blood from a heavily infected animal were taken from the heart and mixed with an equal volume of 1 per cent. citrated Ringer solution. The red cells were then sedimented by slow centrifugation—about 600 revolutions for 5 to 6 minutes. The supernatant fluid contained a uniform suspension of trypanosomes. Definite quantities of the suspensions were added to the sera and the tubes placed at 20° to 25° C. Observations for motility and agglutination were made with the dark field microscope, at various intervals, from 1 to 48 hours. Controls were made with sera from normal animals and from infected animals taken shortly after pyrexial crisis.

In none of the experiments was there any indication of injury to, or even retardation of, the motility during the first six hours. In all of the higher concentrations of serum up to 1.5, actively motile trypanosomes were seen even after an incubation of 48 hours. In general the sera from cured resistant animals reacted in the same manner as did normal sera.

Experiment 2. This experiment was an attempt to apply the Pfeifer reaction to the trypanosomes. Sera from resistant animals were mixed with suspensions of trypanosomes made in the manner described above in the ratio of 5 to 10 parts of serum, respectively, to one part of suspension. The mixtures were injected intraperitoneally into guinea-pigs and, at intervals, material was withdrawn for dark field observation.

There were two variations to this experiment.

Experiment 2a. Two guinea-pigs were each inoculated with 1·0 c.c. serum from cured guinea-pig No. 1 and at the same time 0·1 c.c. of infected blood was injected. A control animal was treated in the same manner with normal serum. Material was withdrawn from the peritoneal cavity at intervals of half, 1, 2, and 18 hours for dark field examination. The trypanosomes remained actively motile, although after 18 hours the stained preparations of the trypanosomes from all the animals showed vacuolations. After an incubation period of 10 to 11 days trypanosomes appeared in the circulation.

Experiment 2b. Sera from three cured resistant animals were pooled and 5 c.c. quantities mixed in varying ratios with trypanosome suspensions. The suspensions were incubated for one hour at 25° C. and then injected into guinea-pigs of approximately the same weight. At intervals similar to those in Experiment 2a, specimens were withdrawn with a capillary pipette and examined under the dark field microscope.

There was no apparent effect on the motility during the twenty-four hours after the injection. Seven to eight days after the injection trypanosomes appeared in the circulation of all of the animals used for the experiment.

This series of experiments indicated that the blood of resistant animals did not contain any trypanocidal antibody which could be demonstrated either *in vitro* or *in vivo*.

Cellular factors in resistance to reinfection.—Immunity is usually considered a two-fold phenomenon—cellular and humoral. In the absence of any demonstrable humoral antibodies we directed our attention to the cellular changes occurring during the infection and subsequent to treatment. It was noted that marked and striking cellular changes occur. The following table (Table IV) gives a summary of white cell counts of a series of animals before and during the infections and before and after treatment. It will be noted that during the infection there is a moderate but uniform leucopaenia with a preponderance of lymphocytes, and that immediately after treatment there is a sharp increase in the total leucocyte count and in the large mononuclears, and a tendency to a reversal of the ratio to normal. This increase is usually greater than normal and the condition persists in all the cured animals for a considerable period.

The bearing of Relapses to Resistance to Reinfection.—It was noted above that the course of the disease in the mule as well as in the experimental animals is intermittent in character ; pyrexia accompanied by the appearance of trypanosomes in the circulation is followed by apyrexial periods of greater or lesser duration. In the rabbit these apyrexial intervals are much greater than in the guinea-pig and occasionally a rabbit may develop a severe type of chronic infection with emaciation, loss of hair, conjunctivitis, keratitis, etc., and yet parasites may be rarely encountered in the peripheral circulation.

The explanation of the relapsing nature of the infection is obscure, but it is undoubtedly bound up in some way with the resistance of the animal host to the invading virus. It appears likely that if not the same, at least similar factors play a rôle in the two phenomena : the resistance to blood invasion and resistance to reinfection ; and that an elucidation of the factors determining the appearance of relapses might throw light on the nature of the immunity.

TABLE IV

Blood picture in Experimental Trypanosomiasis before and after Treatment*

No. of animal		Average Counts							
		During incubation				During infection			
		Total	Polymorphs	Lymphocytes	Mononuclears	Total	Polymorphs	Lymphocytes	Mononuclears
Guinea-pig	104	11,300	65	34	4	8,200	46	52	3
,,	107	8,200	60	35	3	7,000	40	58	2
,,	112	11,100	68	31	3	7,000	52	42	5
Guinea-pig	32	8,000	52	45	3	8,000	37	58	5
,,	33	10,000	60	35	2	10,000	48	49	2
,,	35	9,400	58	40	2	9,200	41	56	3
,,	38	8,400	63	35	2	...	...	...	...
Rabbit	8	8,000	55	43	2	6,500	29	68	3
,,	11	8,000	60	38	2	7,500	54	44	2
,,	18	8,000	58	40	2	7,000	38	60	2
,,	14	9,000	65	33	1	7.400	37	61	2

No. of animal		Average Counts							
		After treatment				Immediately after 'Bayer 205'			
		Total	Polymorphs	Lymphocytes	Mononuclears	Total	Polymorphs	Lymphocytes	Mononuclears
Guinea-pig	104	10,000	58	38	4	10,000	78	16	6
,,	107	7,800	60	39	4	8,000	68	24	7
,,	112	9,700	61	36	3	9,200	45	40	15
Guinea-pig	32	12,000	51	45	4	10,900	38	53	9
,,	33	10,200	63	32	5	8,600	37	54	9
,,	35	...	...	...	...	9,200	38	57	5
,,	38	9,800	53	43	4	12,200	48	47	5
Rabbit	8	11,000	56	42	2	11,000	56	40	3
,,	11	11,500	51	46	3	9,200	58	35	7
,,	18	9,700	47	51	2	10,200	60	34	6
,,	14	10,000	45	52	3	9,900	60	36	4

* The averages are obtained from approximately ten counts made during the infection or after the treatment, and the entire incubation period. It should be noted that during the pyrexial periods there is a marked lymphocytosis which gradually changes to a normal differential during the apyrexial period. In treated animals the maximum total counts are obtained two or three days after the injection of the drug. During the first few days the lymphocytes and large mononuclears predominate, but gradually the cell distribution approaches normal, although the total count may be considerably above the normal average.

Assuming that this interplay of host resistance to virus is associated with the cellular mechanism, we attempted to induce relapses artificially by agents which are known to affect the white cell equilibrium. The work of Bergel (1921) and the results obtained by Nakahara (1922) in his studies on cancer, indicated that oil, particularly olive oil, may produce marked changes in the white cell formula and in the resistance of the host to an invasion of foreign bodies. We consequently used a variety of oils with striking results.

Artificial Production of Relapses.—Relapses were produced at will by the injection of oils, such as cod liver or olive oil, particularly the latter. Ordinary commercial olive oil was employed. The oil was sterilized in the autoclave and injected intraperitoneally. As the protocols indicate, the effect of the oil has been tried on a fairly large series of animals under various conditions and the results have been unfailing in their constancy.

The following are brief extracts of the protocols :

*A. Experiments with guinea-pigs.**

1. *Effect of small doses of olive oil :*

Guinea-pig 13. Infected 1 July ; infection of long duration ; parasites intermittently present in the circulation. On 11 November parasites negative ; 0·25 c.c. sterile olive oil given intraperitoneally. 12 November large number of trypanosomes in the blood ; persistent until 16 November, then negative until 30 November ; 0·25 c.c. oil again given. On 3 December trypanosomes positive and increased steadily in number assuming appearance of continuous infection, until 16 December when treated.

Guinea-pig 26. Infected 22 October ; 26 October blood positive ; remittent infection. On 11 November rare parasites present in the circulation ; given 0·25 c.c. of olive oil i.p. ; on 12 November trypanosomes positive and number increased until 14 November, when the trypanosomes suddenly disappeared, and the blood continued negative.

Guinea-pig 28. Infected 22 October ; parasites infrequently present in small numbers. On 11 November blood negative ; 0·25 c.c. olive oil given intraperitoneally ; on 12 November trypanosomes appeared in the circulation, persisted until 15 November when treated.

2. *Effect of large doses of oil.*

Guinea-pig 12. Infected 19 July ; trypanosomes appeared in the blood on 29 July ; present at irregular intervals for varying lengths of time ; infection unusually long duration. On 11 November trypanosomes were negative ; 4 c.c. olive oil injected i.p. ; 12 November trypanosomes found in the blood. Infection

* The weights ranged from 450 to 500 gms.

positive to 15 November, then negative until 25 November and intermittent. On 30 November another injection of 4 c.c. oil given, 2 December after two days, there was a large increase in the number of parasites and they persisted to the end on 18 December.

Guinea-pig 24. Infected 22 October; 26 October blood positive; intermittent infection. 11 November few parasites in the circulation; injected 4 c.c. olive oil; 12 November large increase in numbers; trypanosomes persisted for seven days and then disappeared. After the blood was negative 11 days, another injection of 4 c.c. given; two days later parasites reappeared in the blood and persisted until 16 December, when treated.

Guinea-pig 27. Infected 22 October, remittent infection. On 11 November blood negative; 4·0 c.c. olive oil injected intraperitoneally. 12 November trypanosomes present in the circulation; persisted until 15 November when animal treated.

Guinea-pig 31. Infected 10 December, trypanosomes in blood 16 December; then negative until 15 January. That day 4 c.c. olive oil injected intraperitoneally; 16 January trypanosomes appeared in the blood and continued present until 17 February when treated.

Guinea-pig 36. Infected 10 December; 18 December blood positive. Examined at intervals until 15 January and no parasites found. On 15 January 4 c.c. olive oil given i.p. On 16 January trypanosomes appeared in the circulation and continued positive until 3 February, when enormous numbers were found in the blood and animal treated.

Guinea-pig 38. Infected 10 December. Blood continually negative. On 15 January 4 c.c. olive oil given i.p.; next day trypanosomes appeared and persisted with two days intermission until 27 January when treated.

B. Experiments with Rabbits.

Rabbit Y. Weight 1390 gms. Infected 5 September. Blood positive 17 September. Parasites rare in circulation; present only five times in two months. On 11 November 5 c.c. olive oil given i.p. On 13 November parasites appeared in circulation, increased in numbers until 18 November; then negative for five days; then increasingly positive until 27 November when the animal died with a heavy blood infection—a most unusual occurrence in rabbits.

Rabbit 4. Weight 1650 gms. Infected 5 November, course of infection irregular; relapses at irregular intervals. On 21 December 10 c.c. olive oil given i.p.; 22 December, trypanosomes present; infection continuous until the animal died.

Rabbit 19. Weight 1250 gms. Infected 10 November, acute conjunctivitis and loss of hair, but trypanosomes constantly negative. On 25 February, 4 c.c. olive oil given i.p.; on 27 February blood was positive and continued so until 3 March, when treated.

The preceding protocols indicate that olive oil injected intraperitoneally into infected guinea-pigs and rabbits was followed usually within twenty-four to forty-eight hours, by an appearance of trypanosomes in the peripheral circulation. The immediate results are not affected by the amount of oil injected; but there seems to be

some relationship between the amount of oil and the persistence, or continued multiplication, of the trypanosomes in the blood. The smaller doses (0·25 c.c.) were followed by a relapse, the persistence of parasites for three or four days, followed by their disappearance for a greater or lesser interval. The larger doses of oil were also followed by the appearance of trypanosomes in the circulation within twenty-four to forty-eight hours after the injection, but the persistence was apparently longer than was the case with the small doses, and in many of the animals the resistance seemed to have been broken down completely. Whenever parasites were present in the body the injection of olive oil served to cause a flare-up and an invasion of the circulation. Rabbits reacted in the same way as guinea-pigs.

Suppression of Resistance in Cured Animals.—Proceeding on the hypothesis that there is a relationship between the resistance to blood invasion and that to reinfection, we attempted to determine whether it is possible to break down the resistance in the same manner as it was possible to produce relapses. Into animals cured from an infection were injected varying doses of olive oil in a manner similar to that used to produce relapses. The results were not uniformly successful, but in a few cases we actually succeeded in breaking down the resistance and calling out an infection which had apparently remained latent. The observations are given in the following protocols :

Rabbit 11. Weight 1750 gms. Injected 26.11.23 ; on 29.11.3, 22 c.c. of bile injected intraperitoneally. Trypanosomes appeared 1.12.23, the rabbit was treated with 0·05 gm. ' Bayer 205 ' per kilo. The blood was rendered negative, and the blood picture changed. On 15 January 4 c.c. oil was given intraperitoneally. On 16 January trypanosomes were found.

Note. The drug had not destroyed the trypanosomes, but rendered the infection latent. A dose of oil caused a flare up of the infection.

Rabbit 7. After three superinfections with negative results (see Table III) this rabbit was given (on 11.11.23) 6 c.c. oil intraperitoneally together with an infective dose of trypanosomes. No trypanosomes appeared in the circulation. On 30.11.23 a similar dose of oil was given intraperitoneally, and on 1.12.23 trypanosomes were found in the blood. From that day trypanosomes appeared intermittently until 23.12.23, when the animal died from the infection.

Note. In this case it appeared that the oil broke down the resistance and called out the infection which was latent for three weeks.

Rabbit 14. Weight 1690. Infected 26.11.23, treated (0·05 gm. per kilo) 16.12.23. Re-infected 20.1.24, results negative. On 25.3.24, 5 c.c. oil together with 0·1 c.c. infected blood given intraperitoneally. No parasites found in the blood. On 14.4.24 another dose of oil given and on 17.4.24 trypanosomes appeared

in the circulation. They then disappeared until 28.5.24, and from then on continued to appear intermittently until the end.

These results could not be duplicated in guinea-pigs treated with ' Bayer 205.' The reason is not apparent, but it would seem that oil is only effective in mobilizing the trypanosomes when the infection is present in a latent state in the body. In any event, the positive results obtained in rabbits increase the presumption that the resistance to reinfection is a temporary phenomenon bound up in some way with the cellular elements and differing from the type of immunity observed in bacterial infection.

IV. DISCUSSION

An outbreak of trypanosome infection in mules in Northern Palestine afforded an opportunity for an experimental study of trypanosomiasis in lower animals, particularly rabbits and guinea-pigs.

The morphology of the parasite, the clinical picture of the disease, and the pathological lesions produced, indicate that the strain belonged to the *T. evansi* group.

In the course of these studies the therapeutic effect of ' Bayer 205 ' and the mode of action of this drug were investigated. The drug proved very effective in clearing up the infection in dogs, rabbits and guinea-pigs ; a single dose of 0·05 gm. per kilo of body weight was sufficient to sterilize the blood in all instances and apparently affected permanent cure in 80 per cent. of the rabbits and guinea-pigs so treated.

The mechanism of the action of the drug is of interest. *In vitro* it has very little trypanocidal power ; even a concentration of 1 : 100 requires twenty-four to forty-eight hours to immobilize the parasites under the condition of the test. *In vivo*, on the other hand, so small a dose as 0·05 gm. per kilo suffices to sterilize the blood of a heavily-infected animal in about sixteen hours. It would seem that the drug is either modified in the animal body and rendered more effectively trypanocidal, or that it acts indirectly on the trypanosomes by increasing the resistance of the host. The fact that a small amount of the drug injected into normal animals affords protection against infection for considerable periods, is further presumptive

evidence that the drug either enters into combination with the tissues and is retained in the body for a long time, or that it produces profound changes in the body leading to increased resistance to infection. Which of these alternatives occur it is difficult to say; it is most likely that both events actually take place.

Experimental evidence is advanced in favour of the view that a real immunity or resistance to infection is set up in the body of animals cured from an infection. Even in normal healthy animals there is a partial resistance to an infection with this strain, as indicated by the relapsing nature of the infection. In the course of the infection, the resistance offered to the invasion of the blood stream is repeatedly broken down (probably by substances liberated by the parasites which continue their activity in the tissues), and the trypanosomes penetrate into the circulation and go through a period of active development. The destruction of the trypanosomes in the circulation leads to a partial immunization resulting in an inhibition of growth and a disappearance of the parasites from the circulation.

This immunity or resistance seems to be associated with the formed elements of the blood. Repeated search failed to reveal any of the usual humoral antibodies (agglutinins, lysins). On the other hand, profound changes occur in the white blood cell formula during the infection, and after the treatment. Moreover, it is possible to break down the resistance artificially by the injection of olive oil, a substance shown by various authors to modify the white cell ratio. This observation, namely, the calling forth of relapses by the injection of olive oil, is analogous to the breaking down of the resistance of mice to transplanted cancer by the injection of large doses of olive oil reported by Nakahara (1923). Since, in the latter instance, the resistance has been shown to be due to a proliferation of cells in the lymphoid tissues, and the suppression to a decrease in the lymphoid cells, there is reason to assume that the same or similar factors are involved in the resistance to trypanosome invasions. In this connection, our observation that the injection of 'Bayer 205' causes a marked increase in the white cell count, particularly the large mononuclears, is significant. The defence of the body against trypanosome infections, and perhaps also to other protozoan infections such as malaria, seems, therefore, to lie in the cellular response rather than in the humoral antibodies.

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